PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

PAUL A. LUCIW, ET AL.

Serial No. 08/083,391

Filed: June 28, 1993

Group Art Unit: 1813

Examiner: M. Woodward

Attorney Docket No. 0035.008

For: HIV IMMUNOASSAYS USING GAG POLYPEPTIDES (AS AMENDED)

DECLARATION

Assistant Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

I, John A.T. Young, do hereby declare as follows:

1. I received my Ph.D. in Human Genetics from the Imperial Cancer Research Fund and University College, London, United Kingdom in 1987 having previously received a B.S. in Biochemistry from the University of Dundee in 1983.

2. I am currently an Assistant Professor, Department of Microbiology and Molecular Genetics, Harvard Medical School. My Curriculum Vitae is attached as Exhibit 1 to my earlier Declaration executed February 18, 1997 and filed in application Serial Nos. 08/442,750 and 08/443,077.

3. I have read and understand Luciw et al. application Serial No. 06/667,501 ('501) filed October 31, 1984.

4. The HIV nucleotide and amino acid sequences provided in the '501 parent application enabled one of ordinary skill in the art in October 1984 to identify antigenic HIV peptides, i.e., peptides containing an antigenic amino acid sequence. To demonstrate this, I performed a hydrophilicity analysis

of the ARV-2 Env and Gag polyproteins according to the Hopp protocol (Hopp 1981, Hopp 1983). The directions in Hopp, together with the hydrophilicity values given in Hopp 1981, permitted a straightforward analysis that was easily within the skill of the art in October 1984. The confirmation of antigenicity was also within the skill of the art in 1984. An antigen could be screened by using it in a prior art immunoassay and testing it with sera of patients known to be infected. This screening process is a technique that is, in fact, disclosed in the Hopp references. My analyses are reported in my prior declarations, Refs. C, D, E and F.

5. I also performed a Hopp hydrophilicity analysis of the ARV-2 pol polyprotein as set forth in the '501 parent application. The analysis identified residues 363-368 as the most hydrophilic region, then residues 695-700 as the second most hydrophilic region and residues 312-317 and 640-645 as the third most hydrophilic regions. These latter residues have been reported to be recognized by a proportion of AIDS patent antisera as demonstrated by Krchnak, *Int. J. Pep. Prot. Res.*, 33:209-213 (1989).

6. The raw data used to generate the hydrophilicity plots for Env, Gag and Pol was obtained according to the method described by Hopp and Woods 1981 *Proc. Natl. Acad. Sci. USA*, 78:3824-3828 and Hopp and Woods 1983 *Molecular Immunology* 20:483-489. Briefly, this method involves assigning each amino acid with a hydrophilic value according to those defined by Hopp and Woods, and then measuring the average hydrophilicity of blocks of six contiguous residues along the entire length of the polypeptide, e.g., the first value obtained would be for amino acids 1 through 6, the second value for amino acids 2 through 7, etc. (See Attachment 1). The hydrophilicity average values for each six residues are then plotted and those with the highest values identified. According to the Hopp and Wood method, the regions with the highest hydrophilicity values are those most strongly correlated with antigenicity.

7. The Hopp hydrophilicity analysis is equally applicable to HIV *Env.*, to HIV *Gag* and to HIV *Pol.* The hydrophilicity values for amino acid residues and the analysis is the same for each case.

8. It was a routine exercise in 1984 to employ 10 to 20-mer synthetic peptides to screen for antigenic sites. Panels of contiguous or overlapping synthetic peptides representing significant portions of whole polypeptides could also be generated by routine methods (e.g., Lerner 1981, PNAS 78:3403-3407). Therefore, with the amino acid sequences provided in the '501 specification, it was routine to test a panel of peptides representing most, if not all of the amino acid residues of HIV proteins to identify antigenic sites by screening the blood of HIV-infected individuals using standard immunoassays, such as ELISA analysis.

9. By October 1984, it was a routine practice to employ synthetic peptides when one desired to work with short polypeptides (e.g., less than about 40 amino acids), whereas expression products were used when one desired to use longer polypeptides. While using peptide fragments generated from polypeptide antigens by limited proteolysis or chemical cleavage was one method for using small polypeptides in the prior art, the use of synthetic peptides was clearly the method of choice. (Sutcliffe 1983, Shinnick 1983).

10. I performed several sequence alignments as shown in Exhibit 1 to demonstrate that while HTLV-I and II are closely related, they have very little sequence homology to HIV. (See Attachment 2). Specifically, the sequence alignments of HTLV-I and II show approximately 68% sequence identity in *Env*, 72% in *Gag*, and 56% in *Pol*. In contrast, the sequence alignments of HTLV-I, II, and HIV displays only approximately 8% sequence identity in *Env*, 14% in *Gag*, and 15% in *Pol*. Most of the amino acids shared between HTLV-I, HTLV-II and HIV are of questionable significance because they are made up

of stretches of only one or two residues. In fact, the longest stretches of identical residues shared between HTLV-I, HTLV-II and HIV are 3 in Gag, 5 in Pol, and 2 in Env. See also Berkhout et al., *Nucleic Acids Research*, Vol. 22, 1705-1711, 1994 for a sequence alignment of HTLV-I and HIV *Pol* polyproteins. The sequence alignment of Figure 2 demonstrates the very same point established by my sequence analysis.

11. Fig. 4 of the '501 specification shows a BstXI site (at the end of the third line) and an NdeI site (on the seventh line). The BstXI site is located within the *Pol* gene and the NdeI site is located downstream of the *Pol* stop codon. The amino acid sequence of the Pol open reading frame shown in the '501 specification is correct between these two restriction enzyme sites.

12. Prior to October 1984, synthetic peptides were routinely used as immunogens, i.e., as reagents that were capable of eliciting an immune response when introduced into an animal host. This fact is clearly documented in two prominent review articles (Shinnick 1983, *Annual Review of Microbiology* 37:425-46; Sutcliffe 1983, *Science* 219:660-666).

13. I agree with the examiner's definitions of the terms "immunogen" and "antigen." Strictly speaking, a polypeptide that is an immunogen is one which is capable of eliciting an immune response, either humoral or cellular, i.e., it is an inducer of an immune response. An immunogen may contain one or more antigenic sites. By contrast, an antigen is the region of that polypeptide that is bound by the highly specific variable regions of an antibody, i.e., it is a target of an immune response.

14. Page 14, line 17 to line 21 of the '501 specification states that:

> "The expression products of the env and gag genes and immunogenic fragments thereof having immunogenic sites may be used for

screening antisera from patients' blood to determine whether antibodies are present which bind to hTLR antigens."

Based on the definition of the term "immunogen," the office action construes this statement to mean that any synthetic peptide being used as a diagnostic reagent would have to be immunogenic, i.e., capable of inducing an immune response by itself. However, this interpretation of the word "immunogenic" fails to take into account this context of its use in the specification. The above teaching clearly instructs someone to test for the presence of antibodies that are pre-existing in patients' blood. These antibodies would have been raised against a complex mixture of viral immunogens including complete polypeptides and also fragments of these polypeptides that resulted from their proteolytic cleavage *in vivo*. It is my firm belief that one skilled in the art would have interpreted the term "immunogenic" set in the context of a diagnostic assay to be "antigenic," i.e., as one that contained binding sites for an HIV-specific antibody. This view is strengthened by the wording of the following paragraph which refers to the peptide as an antigen (page 14, line 28) and by the reference to the peptide as "The antigenic polypeptide of hTLR may also be used...." (Page 14, line 33).

15. By October 1984 it was routine in the art to test polypeptide fragments to determine if they were immunogens by injecting the fragments into an animal host (e.g., rabbit or mouse) and thereafter checking for antibodies raised against the fragment. (Wimmer 1984, *Reviews of Infectious Diseases*, 6 Suppl. 2:S505-509; Bohlen 1984, PNAS 81:5364-5368; Hui 1983, *Science*, 22:1129; Lerner 1984, PNAS 78:3403-3407; Sutcliffe 1983).

16. Based on the teaching and the sequence information provided by the '501 specification, it was within the ability of one skilled in the art in October 1984 to obtain sequences of other HIV clones.

The HUT-78 cell line deposited at the ATCC is identified in Example 1 of the '501 specification. One could make a genomic DNA library of HIV infected HUT-78 cells as illustrated in Example 5 at page 18 in the '501 specification. Because of the high mutation frequency of the virus, this DNA library would contain numerous HIV proviruses with distinct nucleotide sequences. Based on the sequence information in the '501 application, one could screen the library with DNA probes having the sequence of different regions of the HIV genome, e.g., *Env*, *Gag*, or *Pol*. The use of these various DNA probes along with the stated hybridization conditions would lead to the isolation of distinct HIV clones that could then be subjected to DNA sequencing.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 4/5/98 By: John Young

John A.T. Young